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Topical Review

Protein/Protein Interactions (PDZ) in Proximal Tubules

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Introduction

Essentially all cellular functions rely on the correct spatial organization of proteins. In epithelial cells, this is manifested by a separation of the plasma membrane into an apical and a basolateral area, which is required for vectorial transport of solutes, and by the formation of junctional complexes to guarantee barrier function. In this article, we summarize the current knowledge of PDZ protein-based multimeric protein complexes in renal proximal tubular (PT) cells (for definition of the PDZ domain, *see* below), which are involved in the organization of the brush border membrane, the basolateral membrane and the tight junction of PT cells. In addition, we also consider a fourth subcellular structure, the subapical compartment (SAC). This compartment has been ascribed to the recycling of endocytosed proteins and also to the routing of endocytosed material to the lysosomes.

In renal proximal tubules, the majority of filtered solutes and water are reabsorbed by transepithelial transport processes and by paracellular pathways [68]. In addition, PT-cells exhibit an exceptionally high rate of endocytosis to recover small peptides and hormones. On a morphological basis, three different distinct epithelial cells can be distinguished along the proximal tubules. In the S1 segment, brush borders are formed by long microvilli, whereas the appearance of the microvilli in the S2 and S3 segments is less pronounced and varies among different species [37]. Although this longitudinal morphological heterogeneity of PT cells is also reflected by functional differences, in this article, we will not distinguish among proximal tubular segments, i.e., the PDZ-based protein complexes discussed below are assumed to be of uniform nature along the entire proximal tubule.

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The PDZ module

Numerous protein domains have been described that are implicated in protein-protein interactions (see e.g., *www.mshri.on.ca*). Based on sequence similarities between the post-synaptic density protein *P*SD-95, the *Drosophila* junctional protein Disc-large and the tight junctional protein ZO1, one such domain was defined as PDZ. PDZ proteins, among other functions, are primarily thought to organize large functional units such as synapses [55] or tight junctions [19]. The characteristics of PDZ domains have been reviewed recently [14, 15, 28, 55] and are summarized as follows:

In mammals, over 400 different PDZ proteins have been identified and grouped into three major families according to their domain organization: A first family encompasses proteins, which only contain PDZ domains. The second, MAGUK family (membrane-associated guanylate kinases), contains one or more PDZ domains besides a GuK domain (guanylate kinase domain) and a SH3 domain. A third family was defined containing proteins of multiple PDZ domains together with a variable number of other protein domains.

The PDZ domain comprises between 80 and 90 amino-acid residues of which the three-dimensional structure has been basically resolved (*see* http://smart.enbl-heidelberg.de). The building principle is a sandwich structure of 6 β-strands and two α-helices that form a hydrophobic cleft into which a short peptide can be accommodated. As an example, the structural aspect of the CFTR-NHERF interaction has been discussed in detail [38]. In most cases, PDZ domains bind to a C-terminal amino-acid motif. Three different classes of PDZ binding motifs have been recognized. They all include the last four C-terminal amino acids, whose characteristics of interaction can be modulated by more upstream amino acids [55]. Besides the classical canonical determinants, additional PDZ binding motifs have been described and therefore an extended classification has been proposed

[29]. Despite the fact that PDZ domains exhibit high sequence similarities, PDZ-mediated interactions are extremely specific. Based on the nature of the residues residing in helix B1 and the βB strand, a classification into 25 different subgroups was proposed [3]. In contrast to many other protein-protein interactions, PDZ-based interactions are generally regarded as being constitutive and independent of secondary modifications. However, there are no general rules. For example, the association of the β2-receptor or of the multidrug resistance protein MRP2 with PDZ proteins has been shown to be regulated by phosphorylation reactions [22, 24]. In addition to the binding of PDZ domains to C-termini of proteins, the formation of hetero- and homodimers of PDZ proteins via direct PDZ-PDZ domain interactions has been reported. Furthermore, binding of PDZ domains to lipids, such as phosphatidylinositol-4,5-bisphosphate, was demonstrated as well (for review *see* Ref. 55).

PDZ Proteins Expressed in Renal Proximal Tubules

For clarity, we shall assign PDZ proteins of PT-cells to four subcellular regions: the brush border, the subapical compartment, the tight junctional complex and the basolateral membrane (Fig. 1). However, we do not claim that there is no interchange of PDZ proteins between the different regions, e.g., between the brush border and the subapical compartment.

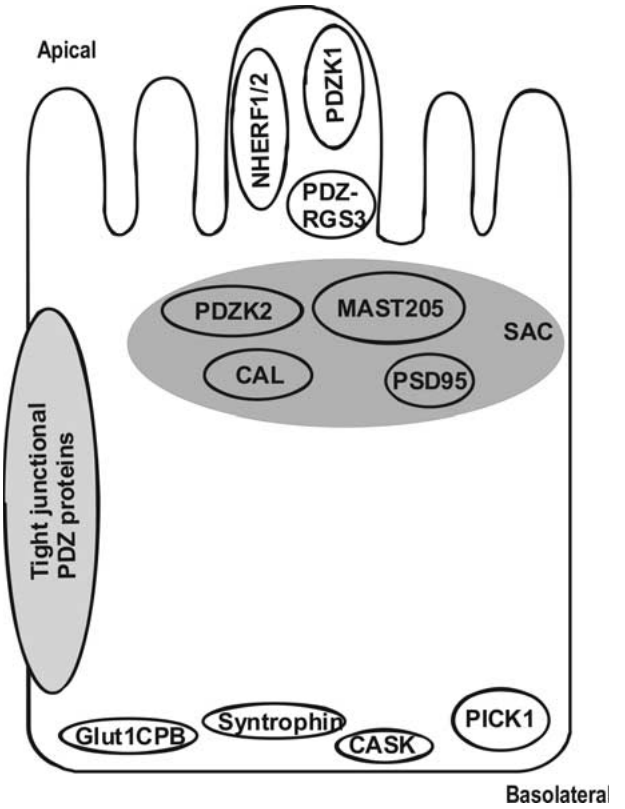

Fig. 1. Putative and established PDZ proteins in the renal proximal tubular cell. PDZ proteins are assigned to the brush borders (apical), to the basolateral site, to the subapical compartment (SAC) and to the tight junction.

Brush Border

A number of PDZ proteins have been shown to reside within the microvilli at the apical pole of PT-cells. These PDZ proteins may act as scaffolds for a variety of apical transporters and may provide anchoring sites for a correct spatial arrangement of apically localized regulatory elements such as kinases, phospholipases and receptors.

NHERF1 (also named EBP50; Ref. 4), originally identified as a regulatory factor of the Na/H-exchanger NHE3 [76], contains two PDZ domains and one MERM (moesin/ezrin/radixin/merlin) binding domain. Activated ezrin binds to the MERM domain and so provides a link of NHERF1 to the actin network [4]. There is good evidence that NHERF1 is localized almost exclusively in the brush borders of all proximal tubular segments [48, 60, 70]. As shown in yeast assays, PDZ domains of NHERF1 also interact with a number of solute transporters, such as the Na/Pi-cotransporter NaPi-lla, the chloride-formate exchanger CFEX, the urate/anion exchanger URAT1, the organic cation transporter OCTN1 [2, 17] and the cystic fibrosis transmembrane conductance regulator CFTR [73]. In addition, interaction of NHERF1 with several components of signalling cascades have been reported as well (*see* below).

NHERF2 (also named E3KARP, Ref. 79), an isoform of NHERF1, contains two PDZ domains in tandem and an ezrin binding site. In heterologous expression systems, such as yeast two-hybrid assays, NHERF2 exhibited a similar interaction pattern with membrane transporters and regulatory proteins as NHERF1 [17]. A difference of the functional roles between the NHERF isoforms may be envisaged because apical expression of NHERF2 appears to be species-dependent: NHERF2 was described in brush borders of mouse renal PT's, but has not been observed in PT cells of rats [70].

PDZK1 (also named NaPi-Cap1, *see* Ref. 18), a protein composed of four PDZ domains, was initially identified by a yeast two-hybrid screen performed against MAP17, a 17 kDa protein that is up-regulated in kidney carcinomas [33, 34, 35]. Before, a shorter version, diphor-1, which lacks the fourth PDZ domain, had been cloned [12]. In PT cells of rats and mice, PDZK1 is restricted to the brush borders [18, 35, 48].

Results from yeast trap assays suggest that PDZK1 associates with the same transporters as NHERF1 [17]. In addition, MRP2 was reported to interact with PDZK1 [34]. Furthermore, the finding

that PDZK1 associates with D-AKAP2 [16], a dual PKA binding protein [26], indicates that PDZK1 targets PKA activity to the brush border of PT cells. A link of PDZK1 to the cytoskeletal network remains unknown.

PDZ-RGS3 was found to interact with NaPi-lla [18]. PDZ-RGS3 is a member of a protein family that has the property to activate GTPases of heterotrimeric G-proteins via RGS (regulator of G-protein signalling) domain an [31]. Therefore, it could be speculated that PDZ-RGS3 could provide a link between transporters and certain signalling pathways.

Subapical Compartment (SAC)

Attempts have been made to define this region as an intracellular organelle [23]. The subapical region of PT-cells is free of mitochondria and contains numerous endocytotic and dense, long-shaped vesicles; the latter likely deliver endocytosed and newly synthesized material to the apical membrane [9]. Also, the SAC can be conceived of as a decision point of membrane traffic, as demonstrated, for example, in the case of the regulation of the NaPi-lla protein by parathyroid hormone (PTH) [53]. Regarding PDZ proteins residing in the SAC, only sparse information is available.

PDZK2 (earlier referred to as NaPi-Cap2) was identified by a yeast two-hybrid screen against the C-terminus of NaPi-lla and has been localized to the SAC [18]. At the amino-acid level, PDZK2 is 26% identical to PDZK1 and contains four PDZ domains in tandem. Thus far, there is no information about a broader interaction palette of PDZK2. It is of interest that the human ortholog, IKEPP, has been identified in the context of the regulation of guanylyl cyclase C [61], suggesting that PDZK2 may act as a similar scaffolder for regulatory components in the PT cell.

CAL (CFTR-associated ligand), a single-PDZ-domain protein, has been localized in the transgolgi region and is thought to regulate the surface expression of CFTR by retention [8]. As CAL also interacts with NaPi-lla (S. Gisler, unpublished results), it could be hypothesized that CAL may regulate surface expression of NaPi-lla or other apically localized transporters as well. The cellular location of CAL in PT cells, however, is not known.

PSD-95 (Post-Synaptic Density protein) is a member of the MAGUK family. PSD-95 was reported to interact with the multiligand endocytic receptor megalin [40]. However, the precise cellular distribution of PSD-95 in PT cells remains to be determined. As megalin is constantly endocytosed and recycled back to the apical membrane [9], this suggests that PSD-95 could orchestrate the trafficking of megalin through the SAC.

MAST205, a microtubule-associated serine/threonine kinase, was originally cloned from testis [71]. Besides a kinase domain, MAST205 posseses one PDZ domain at its carboxy end. A link of the dystrophin/utrophin network to the microtubules via an interaction of the PDZ domain of MAST205 with 2-syntrophin has been described [47]. Recently, an association of MAST205 with NaPi-lla has been revealed on the basis of yeast two-hybrid and biochemical assays [18]. Although the cellular localization of this protein in PT cells is not known, it could be speculated that MAST205 may act as a linker of the Na/Pi-cotransporter to the microtubules. In fact, an involvement of microtubules in the routing of internalized NaPi-lla proteins to lysosomes has been descibed [46].

The Tight-Junctional Complex

In epithelia, paracellular pathways are controlled by the composition of the tight junctions. To date, 12 PDZ proteins have been associated with this macromolecular structure (for review, *see* Ref.19). In addition to the capability to organize the tight-junctional complex, tight-junctional PDZ proteins also scaffold diverse proteins that are involved in signalling pathways for cell growth and differentiation [59]. Not much is known about the expression and cellular localization of PDZ proteins in tight junctions of PT cells.

The MAGUK protein ZO-1 (zonula occludens 1), has been detected in PT cells [20]. ZO-1 associates through the first PDZ domain with the tetraspan proteins claudins. Out of 18 different claudins, claudin 2, 10 and 11 have been found in PT's [1, 32]. As ZO-2 and ZO-3 can be co-precipitated together with ZO-1, it seems likely that ZO-2/3 are present in PT cells as well. Tight junctional PDZ proteins are not only determinants for the permeability characteristics of tight junctions in PT's, but may also be involved in the regulation of transport functions. An example may be the recently described association of the tight-junctional PDZ protein MAGI-3 with frizzled-4, which is part of the Wnt signalling pathway [78]. It is of interest that frizzled proteins have been implicated in the regulation of renal handling of phosphate by the "phosphatonin" frizzled-related protein [56].

The Basolateral Membrane

Most indications of PDZ proteins that reside at the basolateral membrane were obtained from "non-renal" experimental systems. Nevertheless, the PDZ proteins mentioned below may be anticipated to be responsible for the proper basolateral localization of certain transporters.

α-SYNTROPHIN contains a single PDZ domain and two PH (plekstrin homology) domains, which confer the ability to bind lipids or phosphorylated serine or threonine residues. This protein is expressed

in several segments of the nephron, including proximal tubules, where it is localized at the basolateral membrane [45]. α-Syntrophin interacts with AQP4, a water channel that has been assigned to the S3 segment of proximal tubules [69]. In astrocytes of α-syntrophin-deficient mice, AQP4 showed a reversed polarization [54]. However, the impact of α-syntrophin on the polarized distribution of AQP4 in PT-cells has not been analyzed in this model. In HEK cells, it was demonstrated that deletion of the PDZ binding motif (SSV) of AQP4 increased the rate of degradation of the channel and, vice versa, that the expression of α-syntrophin stabilizes the channel in the membrane [54].

CASK (Lin2), another member of the MAGUK family, and VEL1 (Lin7) are PDZ proteins expressed at low levels in the basolateral membrane of PT's [65]. A multiprotein complex containing both proteins recruits Kir2 potassium channels (ESE/AI) in brain [43]. Whether or not CASK and VEL1 fulfill similar tasks in the proximal tubule is unknown.

GLUT1CBP (Glut1 C-terminal binding protein) is a single-PDZ-domain protein expressed in kidney but its precise nephron/subcellular distribution has not been determined. GLUT1CBP interacts with the C-terminal DSQV motif of Glut1, a facilitative glucose transporter detected in the basolateral membrane of proximal tubules, and with α-actinin-1 or other cytoskeletal components [5]. Binding to GLUT1CBP has been suggested to stabilize Glut-1 at the basolateral membrane.

PICK1, a single PDZ domain protein, is able to form homo-oligomers. PICK1 has been proposed to bind (and cluster) several transporters involved in fluid regulation of neurons, such as AQPs and anion exchangers [10]. Although PICK1 was detected in kidney [66], its precise localization and function in renal cells is still an enigma

Functional Impacts of PDZ-mediated Protein-Protein Interactions in PT Cells

With respect to the proximal tubule, direct experimentation to elucidate the functional role of diverse PDZ protein interactions has been difficult. Thus far, only a few knock-out mouse models have been generated that allow one to study the impact of PDZ proteins on proximal tubular function. Information has also been obtained from studies performed with cell cultures, notably OK cells, a cell line originally derived from opossum kidney. One should note, however, that this cell line may differ in a number of aspects from the in-vivo situation [51].

Thus far, there is no clear information about possible roles of PDZ proteins in the sorting of newly synthesized proteins to the apical or basolateral membrane. However, current data provide evidence that PDZ proteins retain different transporters in the different plasma membrane domains. Furthermore, PDZ proteins may scaffold different transporters in microdomains of membranes that may contribute to the regulation of transport processes such as by pH changes or alterations of the ionic situation [52]. In addition, PDZ proteins, directly or indirectly, anchor and orchestrate components of signalling cascades.

Spatial Positioning

At the apical and basolateral membrane of PT cells, most of the described targets for PDZ proteins are transporters. Elucidation of the postulated functions of PDZ clusters became possible with recently generated PDZ protein knock-out mice and with OK cells.

In proximal tubular brush border membranes of NHERF1-deficient mice, the abundance of the type IIa Na/Pi-cotransporter was reported to be decreased [62]. In these mice, kept either on a normal or a low Pi-diet, NaPi-lla accumulates in subapical, intracellular compartments [62, 74]. This suggests that NHERF1 is partially involved in the correct apical localization/positioning of the NaPi-IIa protein. In agreement, impaired apical localization of NaPi-IIa was observed in OK-cells after truncation of its C-terminal PDZ binding motif, TRL, or after overexpression of single PDZ domains [25, 30]. Interestingly, NHERF1 deficiency had no effect on the apical content of NHE3, which is explained, at least in mice, by a compensation with NHERF2 [70].

Studies performed with PDZK1-deficient mice indicated that the apical localization of transporters in PT cells is not dramatically affected. Initial studies performed with PDZK1-deficient mice fed a normal chow showed that the absence of PDZK1 does not alter the abundance of NaPi-IIa or the urinary excretion of phosphate [7, 36].

Involvement of PDZ Proteins in the Regulation of Proximal Tubular Transport

Na/H-exchange and Na/Pi-cotransport represent paradigms for transport functions in PT cells that are regulated by a variety of hormones and metabolic factors (for review, *see* Refs. 53 and 75).

A number of proteins implicated in the hormonal control of Na/Pi-cotransport (NaPi-IIa) and Na/H-exchange (NHE-3) have been described to interact with NHERF1/2: The receptor PTHR1 for PTH [49], the adrenergic β2-receptor [6] and phospholipase β1/2 [27, 64]. The observation that NHERF1 can form homodimers and that the formation of NHERF1 homodimers is regulated [41] indicates that NHERF1 participates in a complex way to orchestrate signalling cascades required to regulate transport functions.

A clone of OKcells, OKH cells, which expresses low levels of NHERF1, revealed direct evidence that NHERF1 assembles an apical, regulatory complex. Transfection of OKH cells with NHERF1 has been shown to restore the PTH-mediated increase of intracellular calcium, which is compatible with the formation of a complex consisting of NHERF1, PTHR1 and PLC-β [50]. How NHERF1 participates in the regulation of the NaPi-IIa protein when the activation of the apical PTHR1 receptor [67] is impaired, is currently under investigation using NHERF1-deficient mice.

Besides the signalling components mentioned above, NHERF1 and 2 anchor protein kinase A (PKA) indirectly via ezrin. The role of the NHERF/ezrin/PKA complex in the regulation of NHE3 has been extensively discussed (for review, *see* Refs. 63 and 75). Results from primary PT-cell cultures of NHERF1-deficient mice are in agreement with the concept that the NHERF/PKA/ezrin/ complex is necessary for the regulation of NHE3 activity by cyclic AMP [11]. In contrast, results obtained with OK cells suggested that NHERF1 is not required for the regulation of the NaPi-IIa protein in response to cyclic AMP [42]. The precise role of NHERF1 in the cyclic AMP-mediated regulation of NaPi-IIa in kidney remains to be determined.

Similar to NHERF1, PDZK1 provides an indirect anchor for PKA by sequestering D-AKAP2, which binds both regulatory subunits of PKA [16, 26]. The precise functional role of the PDZK1/D-AKAP2/PKA complex is currently not understood. Surprisingly, in PDZK1-deficient mice, regulation of NaPi-IIa by PTH or by activation of the PKA pathway was normal [7].

More direct control of transporter/channel function by PDZ proteins has been observed for the chloride permeability of CFTR. Interaction of recombinant NHERF1 with CFTR increases the open probability of CFTR and phosphorylation of NHERF1 influences this channel modulation [57]. Similarly, a potentiation of CFTR channel activity was reported by the interaction of CFTR with PDZK1 [72].

Are NHERF1 or PDZK1 needed for the rapid and/or chronic adaptation of the Na/Pi-cotransporter NaPi-IIa? Dietary content of phosphate robustly regulates the abundance of the NaPi-lla protein in PT-cells, yet, the precise mechanisms are not known. Recent data obtained with NHERF1-and PDZK1-deficient mice indicated that neither NHERF1 nor PDZK1 is important for the regulation of NaPi-IIa by dietary content of Pi, albeit apical expression of NaPi-IIa after a low Pi-diet was slightly impaired in NHERF1-deficient mice [7, 74]. In contrast to the in-vivo findings, up-regulation of NaPi-IIa by a low-Pi medium was abrogated in primary cultures derived from NHERF1-deficient mice [11].

Regulation of PDZ Interactions by Phosphorylation

Until now, only a few examples have been reported that PDZ interactions can be modulated by phosphorylation reactions either of the PDZ protein itself or of amino acids close or within the PDZ binding motif. It may be envisaged that phosphorylation reactions may allow an on-off mode of PDZ interactions. In PT cells, the necessity for an on-off mechanism of a PDZ interaction is best illustrated by the down-regulation of the Na/Pi-cotransporter NaPi-IIa in response to PTH. NaPi-IIa, which is localized along the entire length (>1 μm) of the microvilli is only internalized at the base of the microvilli, the intermicrovillar clefts [77]. As upon a stimulation of PTH receptors the localizations of NHERF1 and PDZK1 are not altered (N. Deliot, *unpublished results*), it is assumed that the affinity of NaPi-IIa to the PDZ domains is decreased by PTH in order to enable a higher diffusional mobility of NaPi-IIa. In analogy, increased diffusional mobility has been demonstrated for CFTR after truncation of its PDZ binding motif [21].

NHERF1, but not NHERF2, is constitutively phosphorylated [58]. The role of phosphorylation of NHERF1 has been extensively studied in the context of the PKA-mediated inhibition of NHE3 [75]. Recent results obtained in the authors' laboratory by in-vitro experiments using mouse kidney slices showed that PTH induced an increase of NHERF1 phosphorylation. Augmented phosphorylation of NHERF1 was also observed after individual activation of PKA and PKC, respectively (N. Deliot, *unpublished results*). Interestingly, cyclic AMP-dependent regulation of NHE3 in OK-cells was not paralleled by an alteration of NHERF1 phosphorylation [39]. Similarly, in kidney slice experiments, constitutive and regulated phosphorylation of PDZK1 was observed (N. Deliot, *unpublished results*).

Modulations of PDZ interactions by phosphorylation reactions have been demonstrated in the following cases: i) Phosphorylation of a serine residue within the C-terminal PDZ binding motif SLL of the β2-adrenergic receptor inhibits the interaction of the receptor with NHERF1 [6]; ii) Overlay experiments demonstrated that phosphorylation of the serine residue from the PDZ binding motif STKF of MRP2 has a positive effect on the interaction with NHERF1 [24]; iii) Phosphorylation of the PDZ domain 2 of NHERF1 (on Ser-162) in response to activated PKC resulted in an inhibition of the interaction between NHERF1 and CFTR [57].

Is the Abundance of PDZ Proteins in PT Cells Regulated?

Sparse information is available about the regulation of the abundance of PDZ proteins in PT-cells.

Transcriptional regulation of NHERF1 by estrogen and up-regulation of PDZK1 in renal carcinomas have been reported [13, 34].

The scaffolding functions of PDZ proteins discussed above may suggest that under conditions that lead to significant up or down-regulation of a specific transport function, the abundance of PDZ proteins may be altered as well. One such potential situation is the intake of a low phosphate diet, which results in up-regulation of the NaPi-lla Na/Pi-cotransporter [44]. However, despite the robust interaction of NaPi-IIa with NHERF1 and PDZK1 as observed in biochemical assays, a parallel up-regulation of neither NHERF1 nor PDZK1 was observed in the authors' laboratory [48]. In contrast, up-regulation of the PDZK1 protein by a low Pi-diet has been reported by others [11, 74].

Summary

Using kidney cDNA libraries and single proximal tubular proteins as baits, the yeast two-hybrid technology resulted in the description of numerous potential PDZ-based protein-protein interactions. Many of these have been confirmed by biochemical in-vitro assays. In order to assign cellular functions of such proteins, it is first mandatory to define the precise distribution and localization in PT cells of each canditate protein. This prerequisite has been assessed only for a few of the PDZ proteins mentioned in this article. In addition, it remains to be deciphered how the interactions of the identified PDZ proteins are modulated, for example, by phosphorylation reactions or by other posttranslational modifications. As mentioned in this article, PDZ knock-out mouse models may be of help to elucidate the physiological and pathophysiological functions of a particular PDZ interaction. However, current data indicate that, despite the robust interactions observed in in-vitro assays, ablation of a certain PDZ protein does not necessarily result in an expected phenotype, probably due to functional compensation by other PDZ proteins. Therefore, it has to be assumed that a large redundancy of known and as yet unidentified PDZ proteins exists in proximal tubular cells.

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